

Method for obtaining an anti-tumor substance from even-toe hoofed mammals

5       The invention relates to a method for obtaining an anti-tumor substance from even-toe hoofed mammals (artiodactylous animals) having leucosis.

      The international publication WO 00/40256 relates to the tumor inhibiting effects on different types of tumors of the blood plasma obtained from patients suffering from acute leukemia. In the specification several sets of experiments were disclosed, wherein the  
10   applied substance resulted in an improvement by 20% compared to the control group. Such an improvement constitutes the lower limit of what can be qualified as significant.

      In my Hungarian patent application P 0002597 I have recognized that from the lipid-free blood plasma fraction of even-toe hoofed mammals (artiodactylous animals) having leucosis a substantially more efficient tumor-inhibiting effect can be obtained than from  
15   what can be obtained from leukemic blood, and a method was disclosed for obtaining the plasma component responsible for the effect.

      The effect was confirmed by the experiments carried out with animals having different types of tumor disclosed in the cited patent application.

      In the published international patent application WO 02/07739 claiming the priority  
20   of the cited Hungarian patent application further experiments with animals were disclosed for verifying the tumor-inhibiting effect, which related to further tumor types, and the effect was demonstrated in case of different ways of administering the active substance (intramuscular, subcutane, intraperitoneal and per os).

      In view of the outstanding tumor-inhibiting effect, both the preparation method and  
25   efficiency of obtaining the active substance have great significance. Besides the possibility of obtaining the material from animal blood one has to examine all other ways by which one can arrive at the same substance.

      The object of the invention is to provide a more efficient method for obtaining the active substance described in the cited patent applications.

30       I have recognized that the concentration of the tumor-inhibiting substance (as an immune antibody) is changing in the blood of the animal during pregnancy, furthermore the substance appears in the milk immediately following the delivery. The milk provided

WO 03/059364

PCT/HU03/00004

2

during the first feeding after delivery and during a few number of subsequent feeding is referred to as colostrum.

In the animal organism the immune system makes sure that the newborn obtains the available antibodies of the immune system of the suckler by consuming the colostrum during the first few lactation in a concentrated way, whereby it will be resistant against outer infections. In the blood of the suckler the concentration of the antibodies will thereby decrease in the period just following the delivery, and these materials will be concentrated in the colostrum. This phenomenon can be observed in the first few weeks of lactation.

According to the first aspect of the invention, the colostrum of even-toe hoofed mammals (artiodactylous animals) primarily cattle (cows) that have leucosis is utilized for obtaining the active substance responsible for the tumor-inhibiting effect.

According to the second aspect of the invention the fact is utilized that the concentration of the antibodies in the blood of the pregnant animal increases during the medium and last third of pregnancy, and the active substance is taken from the blood of donor pregnant animals being in such state of pregnancy. The most preferred animals are cattle and sheep. The utilizable period ends because in the last days of pregnancy and during the first few days of lactation the antibody level in the blood decreases and it increases in the colostrum to a high level.

The active substance is obtained from the colostrum by using a separation method similar to the one disclosed in the above referred patent application, wherein the starting material was the blood plasma of the animal.

The separation method comprises preferably the following steps:

- 1) The colostrum is shaken with a 1:1 mixture of i-propyl alcohol and chloroform of identical volume on room temperature through 8 hours in a shaker machine
- 2) The material is centrifuged at a speed of 10000 rev/min through 20 minutes in a cooled state.
- 3) The floating upper layer and the medial crust layer are separated (the organic phase is spilled), and the rest of the material is diluted with the addition of a 1: 1 mixture of chloroform and benzyl alcohol to take the original volume and shaken through 8 hours with a shaker machine.
- 4) The material is stored through 10 hours at a temperature of +2-4 °C.

WO 03/059364

PCT/HU03/00004

3

- 5) The material is centrifuged just as in step 2 and the organic phase is spilled.
- 6) The floating upper layer is deep-frozen and freeze-dried and by means of a physiologic saline solution the required concentration is adjusted. This product was used for efficiency examination of tumorous mice and it has proven biologically active.
- 7) The central, jelly-like crust layer (in step 3) is frozen and freeze-dried, and the treatment is continued as in step 6. The so-obtained material was biologically tested and it was efficient on tumorous mice.
- 8) The samples obtained in steps 6 and 7, respectively were united and this material was regarded as final product.

By using identical doses of the so isolated active substance the animal experiments disclosed in the cited Hungarian and international patent applications were repeated, but the active substance was given always through the mouth (per os).

- 15 The experiments resulted in identically favorable results in case of all examined types of tumors as in case of the previous experiments carried out with the active substance taken from the blood plasma, which is understandable, since the active substances were substantially identical.

- 20 By using the method according to the invention substantially higher amounts of substance can be obtained as if the material had been taken from the blood of the same animal, furthermore the utilization of the colostrum does not cause harm to the animal.

For illustrating the quantitative proportions it should be noted that a cow with average weight can supply about 20 liters of colostrum every day in the first period following delivery that can last even up to 3-4 weeks.

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